PCT WORLD INTELLI

INTERNATIONAL APPLICATION PUBLISH

WO 9603141A1

(51) International Patent Classification 6 :
A61K 38/17, 39/395 // C07K 19/00 A1

(43) International Publicati n Date: 8 February 1996 (08.02.96)

(21) International Application Number: PCT/EP95/02788

(22) International Filing Date: 15 July 1995 (15.07.95)

(30) Priority Data:
94111455.5 22 July 1994 (22.07.94) EP
(34) Countries for which the regional or international application was filed: AT et al.

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(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING A CHIMAERIC TNF BINDING PROTEIN

(57) Abstract

The present invention is directed to the use of a chimaeric TNF binding protein which comprises a soluble part of the human p55- or the p75-TNF-receptor and all or parts of the constant domains of the heavy or light chain of human immunoglobulin or of a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment of autoimmune diseases.

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Pharmaceutical compositions comprising a chimaeric TNF binding protein

The present invention is directed to the use of a chimaeric TNF binding protein which comprises a soluble part of the human p55- or the p75-TNF-receptor and all or parts of the constant domains of the heavy or light chain of human immunoglobulin or a pharmaceutically acceptable salt thereof for the method of treatment autoimmune diseases, which are frequently associated with inflammatory processes, e.g. rheumatoid arthritis, juvenile onset type I diabetes mellitus, systemic lupus erythematosus, thyroiditis, especially multiple sclerosis and for the preparation of a pharmaceutical composition for the treatment thereof.

Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system (CNS), thought to originate in an autoimmune disorder, with components of myelin as the target. Support for the autoimmune origin of MS is provided by the fact that myelin basic protein (MBP) reactive T lymphocytes are found in man which are phenotypically similar to MBP-specific encephalitogenic T cells in experimental allergic encephalomyelitis [Sun, Acta. Neurol. Scand. Suppl. 142, 1-56 (1993); Voskuhl et al., Autoimmunity 15, 137-143 (1993)], and there is evidence that the frequencies of MBP reactive T cell clones in MS patients is elevated [Allegretta et al., Science 247, 718-721 (1990)]. In addition, various experimental autoimmune encephalomyelitis animal models have been developed which mimic pathophysiologic aspects of human MS (see below). However, there are also environmental factors controling MS incidence as evidenced by the existence of geographically well defined endemic areas.

MS is characterised by focal accumulations of inflammatory cells, edema and demyelination in CNS tissue. It is an old finding that at autopsy more

focal lesions are found than would have been predicted from the clinical status.

Typically, the lesions start as a perivenular infiltrate of mononuclear cells in white matter with ensuing edema. The infiltrating cells appear to mediate the selective destruction of myelin sheaths leaving the axons intact. In the further progression, astrocyte proliferation leads to a gliotic scar, the so-called MS plaque, of 0.1 to several cm diameter. The clinical course of MS may be remitting/relapsing with a wide range in the duration of the disease free interval, or chronically progressive suggesting that the underlying pathophysiologic processes are under heterogeneous control.

Clinical pathology studies have revealed the presence of TNF producing cells in MS lesions. The majority of the TNF positive cells were morphologically classified as reactive fibrous astrocytes, and double immunostaining experiments confirmed that most reactive cells were astrocytes, the rest being monocyte / macrophages [Hofman et al., J. Exp. Med. 170, 607-612 (1989)]. An independent study confirmed the positive immunoreactivity for TNF in astrocytes and monocytes in MS plaques, and also investigated lymphotoxin (LT) immunoreactivity. Interestingly, in additon to the expected lymphocytes, microglial cells were found positive for LT [Selmaj et al., J. Clin. Invest. 87, 949-954 (1991a)]. Furthermore, investigations of the cytokine mRNA expression in MS lesions by in situ hybridisation show that high levels of TNF, IL-6 and IFN-γ (and other cytokine) mRNAs are present in all inflammatory perivascular lesions. The levels of TNF, IL-6 and IFN-γ mRNAs were particularly high in those lesions which had strong demyelination [Woodroofe and Cuzner, Cytokine 5, 583-588 (1993)].

A direct cytotoxic effect of TNF, but not of IFN-γ, IL-2, T-cell supernatants or antigalactocerebroside antiserum, on oligodendrocytes in myelinated cultures of mouse spinal chord tissue has been reported [Selmaj and Raine, Ann. Neurol. 23, 339-346 (1988)]. TNF treatment of these cultures caused oligodendrocyte necrosis; some nerve fibers progressed to demyelination.

TNF concentrations in serum and cerebrospinal fluid (CSF) of MS patients have been measured by a number of investigators. There is a consensus that a significant percentage number of MS patients has elevated TNF in CSF, and much less so in serum. The concentration of TNF does not directly relate to CSF cell content suggesting that CSF TNF content might reflect

TNF production in and around MS lesions, especially in view of the frequent paraventricular location of lesions.

Animal studies in experimental encephalomyelitis (EAE) have significantly shaped the views on the autoimmune origin and the role of cytokines in human MS. EAE is induced either by active sensitisation with myelin antigens or by adoptive transfer of specific T cell clones into a syngeneic, susceptible host animal. The finding that the experimental induction of an immune response to myelin antigens leads to diseases with similarities to human MS is an important argument for an autoimmune origin of MS. The role of TNF in the establishment and the effector mechanisms responsible for the the characteristic pathology of the disease is demonstrated by the protective efficacy of anti-TNF monoclonal antibody therapy [Ruddle et al., J. Exp. Med. 172, 1193-1200 (1990); Selmaj et al., Ann. Neurol. 30, 694-700 (1991b)]. The elimination of cells producing TNF (and other cytokines) similarly suppresses EAE [Huitinga et al., J. Exp. Med. 172, 1025-1033 (1990)]. Further support for an important role of TNF in EAE is provided by the finding that encepalitogenicity in adoptive transfer strongly depends on the TNF and LT production capability of various T cell clones, all recognising the same MBP peptide [Powell et al., Intern. Immunol. 2, 539-544 (1990)].

The use of soluble parts of the human p55- and p75-TNF-receptor for the treatment of experimental encephalomyelitis (EAE) in rats has already been described in EP 512 528. However, so far no data regarding the protective effect of chimaeric TNF binding proteins and pharmaceutically acceptable salts thereof have been reported. Therefore the use of a chimaeric TNF binding protein which comprises a soluble part of the human p55- or the p75-TNF-receptor and all or parts of the constant domains of the heavy or light chain of human immunoglobulin or a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment of autoimmune diseases, e.g. multiple sclerosis, especially, wherein the chimaeric TNF binding protein comprises the soluble part of the human p55-TNF-receptor is an object of the present invention. A furthermore preferred embodiment of the present invention is such a use wherein the chimaeric TNF binding protein comprises all domains except the first domain of the constant region of the heavy chain of human immunoglobulin which means that the soluble part of the human p55-TNF-receptor is fused at its C-terminal end to the hinge region of the

immunoglobulin heavy chain. Such immunoglobulin can be either IgG, IgA, IgM or IgE, especially IgG, like IgG1 or IgG3. The term "a soluble part of the human p55- or the p75-TNF-receptor means in the context of the present invention either the complete extracellular domain of one of these receptors or a part thereof which still binds human TNF, e.g. in case of the p55-TNF-receptor the extracellular domain lacking the first 12 N-terminal amino acids.

Furthermore it is an object of the present invention to provide a method of treating an autoimmune disease in a mammal comprising administering to said mammal a therapeutically effective amount of a chimaeric TNF binding protein or a salt thereof as defined above which ameliorates the effects of such autoimmune disease, specifically such a method wherein the autoimmune disease is multiple sclerosis.

The preparation of the chimaeric TNF binding proteins to be used for the purpose of the present invention is described in detail in European Patent Application Publication No. (EP) 417 563 and Loetscher et al., J. Biol. Chem. 266, 18324-18329 (1991). Furthermore TNF binding proteins or parts thereof as described in the following patent publications can be used for the preparation of such chimaeric TNF binding proteins : EP 308 378, EP 422 339, GB 2 218 101, EP 393 438, WO 90/13575, EP 398 327, EP 412 486, WO 91/03553, EP 418 014, JP 127,800/1991, EP 433 900, U.S. Patent No. 5,136,021, GB 2 246 569, EP 464 533, WO 92/01002, WO 92/13095, WO 92/16221, EP 512 528, EP 526 905, WO 93/07863, EP 568 928, WO 93/21946, WO 93/19777, EP 417 563 and WO 94/06476.

The term chimaeric TNF binding protein as used throughout the specification comprises also such proteins in which any amino acid of the immunoglobulin part or the TNF binding part has been deleted or substituted by one or more amino acids or one or more amino acids have been added as long as the TNF binding part still binds TNF and the immunoglobulin parts shows one or more of its characteristic properties. In case the immunoglobulin part consists of all domains including the hinge region except the first domain of the constant region of the heavy chain such hinge region may lack the first five N-terminal amino acids. Such chimaeric TNF binding protein muteins can be prepared by methods known in the art and described e.g. by Sambrook et al. in "Molecular Cloning" (second edition 1989, Cold Spring Harbor Laboratory Press, New York) or in one or more of the above mentioned patent publications.

Furthermore the chimaeric TNF binding protein of the present invention can be also used in modified form, e.g., when coupled to chemical entities without altering its basic biological activity. A preferred and well known modification is the coupling to water soluble polymers, e.g., polyethylene glycols or polypropylene glycols, within a wide range of molecular weight of, e.g., from 500 to 20'000 daltons. This leads to protected proteins, which could be substantially non-immunogenic. Several modes of coupling the polymer to the protein via different linkers are available in the state of the art and described, e.g., in general in "Perspectives in Bioconjugate Chemistry", edt. by C.F. Meares, American Chemical Society, Washington 1993, and specifically, e.g., in U.S. Patent No. 4,179,337.

Furthermore the chimaeric TNF binding proteins of the present invention can be in the form of a pharmaceutically acceptable salt. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids such as, for example, hydrochloric acid or sulfuric acid and salts with organic acids such as, for example, acetic acid or oxalic acid.

The chimaeric TNF binding protein to be used for the purpose of the present invention is preferably administered parenterally by injection, although other effective administration forms, such as intraarticular injection, or transdermal iontophoresis are also possible. One preferred carrier is physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used. The primary solvent in such a carrier may be either aqueous or non-aqueous in nature. In addition, the carrier may contain other pharmacologically acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmacologically acceptable excipients for modifying or maintaining the stability, rate of dissolution, release, or absorption of the chimaeric TNF binding protein. Such excipients are those substances usually and customarily employed to formulate dosages for parental administration in either unit dose or multi-dose form.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or in dehydrated form, e.g. as lyophilized powder. Such formulations may be stored either in a ready to use form or a form requiring reconstitution immediately prior to administration. The preferred storage of such formulations is at temperatures at least as low as 4°C and preferably at -70°C. It is also preferred that such formulations are stored and administered at or near physiological pH.

A possible dosage range for the treatment of multiple sclerosis may be between about 0.001-5.0, preferably 0.1-5.0, more preferably 0.1-2.0 mg per kg of patient body weight per 1-4 weeks administered in a single dose and most preferably between about 0.1-2.0 mg per kg of patient body per 1-4 weeks and administered in a single dose of a chimaeric TNF binding protein which comprises a soluble part of the human p55-TNF-receptor and all or parts of the constant domains of the heavy or light chain of human immunoglobulin or a pharmaceutically acceptable salt thereof, preferably wherein such chimaeric TNF binding protein comprises all domains except the first domain of the constant region of the heavy chain of human immunoglobulin such as IgG, IgA, IgM or IgE and most preferably wherein the human immunoglobulin is IgG, especially IgG1 or IgG3. The frequency of dosing and the optimal dose will depend on pharmacokinetic parameters of the chimaeric TNF binding protein in the formulation used.

Regardless of the manner of administration, the specific dose is calculated according to the approximate body weight of the patient. Further refinement of the calculations necessary to determine appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by the man skilled in the art.

Examples

Example I

Acute EAE model (AHH/R rats)

Female AHH/R rats [Bloxham et al., J. Pharmacol. Exp. Ther. 252, 1331-1340 (1990)] were inoculated with emulsion (0.05 ml) containing guinea pig spinal cord and Freunds Complete Adjuvant (1:1) containing Mycobacterium tuberculosis (Difco Laboratories, Detroit, MI, USA) (10mg/ml) into the planter surface of both hind paws. Animals were weighed and evaluated

daily for neurological signs according to a 4-point score system (clinical score): 0=normal; 1=flaccid tail; 2=partial hind limb paralysis; 3=full hind limb paralysis; 4=tetraplegia. Groups of rats were dosed with a construct of the extracellular domain of the human p55-TNF-receptor (first 182 N-terminal amino acids of the receptor) fused to the hinge region of human IgGγ1 heavy chain and expressed in CHO-cells ("fusion construct") (i.p.) or with phosphate buffered saline (PBS) vehicle only. The administration of the encephalitogenic suspension in rats resulted in an onset of neurological symptoms (day 10) which subsequently peaked on day 14. The neurological symptoms were accompanied by a significant weight loss. Treatment with the fusion construct (1, 5, and 10 mg/kg, i.p., dosed daily, post-induction of EAE, days 8-14), significantly reduced the symptoms of EAE and the changes in body weights in a dose dependent manner [Figure 1 shows the mean clinical score values of 10 animals in each group from day 10 to 21 and Figure 2 shows the corresponding mean body weight from day 8 to 20; p-values were determined by the Mann-Whitney-U-test (see statistical Methods in Biology, Bailey N.T.J., Hodder and Staughton, London)]. In a second series of experiments the fusion construct (10 mg/kg, i.p.) adminsitered post-induction throughout the disease "window" (days 8-20) again significantly reduced the symptoms of EAE and the changes in body weight up to day 14 [Figure 3 shows the mean clinical score values of 10 animals from day 10 to 20 with p-values determined by the Mann-Whitney-U-test, s.a. and Figure 4 shows the corresponding mean body weight with p-values determined by the unpaired-T-test (see Statistical Methods in Biology, s.a.)]

Example II

Chronic EAE model (Biozzi mice)

Inbred Biozzi Selection I AB/H mice [Baker et al., J. Neuroimmunol., 28, 261-270 (1990)] were maintained on standard pelleted diet and water ad libitum. Male mice were inoculated subcutaneously on the abdomen in two sites with 0.3 ml (total 0.6 ml) of an emulsion containing 6.6 mg/ml freeze-dried Biozzi spinal cord in phosphate-buffered saline (PBS) and 60 mg heat-killed mycobacteria [Mycobacterium tuberculosis H37Ra and M. butyricum (8:1); Difco, s.a.] in 0.15 ml of Freund's incomplete adjuvant (Baker et al., s.a.). The injections were repeated 7 days later. Animals were weighed and evaluated daily for neurological signs according to a 5-point score system: 0=normal; 1=limp tail; 2=impaired righting reflex (IRR); 3=partial hind limb paralysis, 4=complete hind limb paralysis; 5= death. Groups of mice were

dosed with the fusion construct (see Example I) (i.p.) or phosphate buffered saline (PBS) vehicle. In the chronic EAE model, the Biozzi mice, the incidence of acute disease in the vehicle group was 90 %, with neurological symptoms appearing n day 14 post-induction of EAE. Pretreatment with the fusion construct (7 mg/kg, i.p., dosed every 3 days throughout the experimental protocol), reduced the number of animals exhibiting paralysis (Nos. 1-4 in 5-point score system) during both the acute phase (3/9) and relapse phase (1/7) of the disease as compared to the controls (9/10 and 6/8, respectively;) [see Table 1 and Figure 5 which shows in Figure 5a the control experiment only with pure saline solution ("vehicle") and in Figure 5b the situation with the fusion construct wherein the bars give the clinical score values and the curve is an indicator for the change in body weight (g)]. In a subsequent dose response study where the fusion construct (1-14 mg/kg, i.p.) was administered post-induction of the disease (days 10-30) beneficial effect was also demonstrated, 7 mg/kg being the minimum effective dose.

Example III

General methods

Animals, reagents, and cell lines

Inbred Lewis rats, 120 to 150 g in weight, were obtained from Charles RIVER WIGA (Sulzfeld, BRD). Guinea pig myelin basic protein (MBP) was isolated from whole brains [Eylar et al., J. Methods Enzymol. 32B, 323 (1979)]. C1, the encephalitogenic peptide of MBP (amino acids 68-88) was purchased from Peptide Products (Porton Down, United Kingdom). Bovine S100β protein was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Protein purified derivative from M. tuberculosis (PPD) was obtained from Statens Seruminstitut (Kopenhagen, Denmark). A monoclonal antibody against myelin oligodendrocyte glycoprotein [anti-MOG Mab 8-18C5, Schlüsener et al., J. Immunol. 139, 4016 (1987)] was produced from hybridoma supernatants and purified on protein A-sepharose beads (Sigma).

Immunization

Lewis rats were immunized into the hind footpads with either MBP (100 µg/rat), C1 (50 µg/rat), or S100β protein (50 µg/rat) emulsified in Freund's adjuvant (Gibco BRL AG, Basle, CH) completed with 4 mg/ml M. tuberculosis (H37Ra, Difco, Detroit, USA).

Antigen-specific T cells

T cell lines used in this study were specific for guinea pig MBP and/or C1 (F8, C1-C9, C1, LMBP) or bovine S100β protein, a non-myelin, calcium binding protein (LS1). Two different protocols were used to establish antigen-specific T cell lines. Ten days after immunisation a single cell suspension was prepared from the draining popliteal, inguinal, and para-aortic lymph nodes. The primed cells were either cultured at a concentration of 10^7 cells/ml (5 ml, petri dish) and antigen (10 µg/ml) to establish bulk T cell lines (LS1, C1, LMBP) or alternatively serially diluted to provide a large number of pauciclonal T line cells (F8, C1-C9). In the latter case, lymph node cell suspensions were cultured in 96-well round-bottom microtiter plates at cell numbers ranging from 2×10^5 to 100 cells/well. The cells were supplemented with antigen and irradiated (4,000 rad) syngeneic thymus cells as source of antigen presenting cells (APC's) to give a final cell number of 2×10^5/well in Eagle's medium (EA) enriched with L-asparagin (36 mg/ml), L-glutamine (2 mM), sodium pyruvate (1 mM), nonessential aminoacids (1 % v/v), penicillin (100 U/ml), streptomycin (100 mg/ml; Gibco) and autologous rat serum (1 %). After 72 hrs the medium was removed and the remaining T cells expanded for further 7-10 days in media supplemented with IL-2. The T cells were then restimulated with antigen in the presence of 2×10^5 irradiated APC's. After visual inspection of the cultures, those positive for growth were expanded in the presence of IL-2. Activated blastoid lymphocytes generated in bulk cultures were collected from the interface of Lymphoprep-gradients (density 1.077 g/ml, Nycomed Pharma AS, Oslo, Norway). The resting T cells were then restimulated in the presence of irradiated APC's (T cell to thymus cell ratio: 1:30). The cycle of propagation in the IL-2 containing medium and restimulation with antigen plus APC's was then repeated until stable T cell lines were established.

Specificity assay

To evaluate T cell specificity a standard T cell proliferation test was used. T cells (2 x 10^4/ml) were restimulated in flat-bottom microtiterplates (200 µg/well) in the presence of APC's (6 x 10^5/ml) and the relevant antigens MBP, C1 or S100β (20 µg/ml), the non relevant antigen PPD (10 µg/ml) and ConA (2.5 µg/ml). After 72 hrs of culture the cells were harvested following a 16 hrs pulse with 1 µCi/well tritiated thymidine (^{3}H-dT; 2 Ci/mmol; Amesham-Buchler, Braunschweig, BRD). The radiolabelled cells were collected on glassfiber filters and the radioactivity associated with the washed and dried filters was quantified with a Matrix 96 counter (Canberra-Packard, Frankfurt, BRD).

Active EAE

Active EAE was induced in rats following immunization with MBP or C1. The weight and clinical score were monitored on a daily basis by clinical inspection. Clinical disease was evaluated as follows: 0 = normal, 1 = tail limpness, 2 = paraparesis with clumsy gait, 3 = hind limb paralysis, 4 = hind and fore limb paralysis, 5 = death. The study was done in a blinded fashion. That is, the observer was unaware of the protocol.

Passive EAE

Passive EAE was induced in syngeneic rats following intraperitoneally injection of freshly antigen-activated T cell blasts (5-7 x 10^6/rat). If S100β specific T cells were used to induce EAE the rats received 10^7 cells followed by a single dose of an Mab 8-18C5 (3-5 mg/rat) at day five after the cell transfer. The weight and clinical score was monitored daily as described above. At appropriate time points animals were sacrificed and used for conventional central and peripheral nerve histology.

Treatment of EAE

Activ or passively induced EAE was treated by repeated or single intraperitoneal injections with 10 mg/kg of the fusion construct (see Example I).

FACS-Analysis

Mouse anti-rat T cell-specific monoclonal antibodies (MAb) pan-T (W 3.13), CD4 (W 3.25), CD8 (OX-8), aβ-TcR [R73, Hünig et al., J. Exp. Med. 169, 73

(1989)] were purified from hybridoma supernatants on protein G-sepharose beads (Sigma, Taufkirchen, BRD) or purchased from Camon (Wiesbaden, BRD). For TcR Vβ isotypespecific MAbs B73 (V 8.5), G101 (Vβ 10), and R78 (Vβ 8.2) see Torres-Nagel, Immunogenetics 37, 305 (1993). Labelling of specific MAbs was detected using a DTAF-labelled F (ab')2 fragment of goat anti-mouse IgG (H+L chain). Immunofluorescence of viable cells excluding propidium-iodate (10 µg/ml, Sigma) was measured with a FACScan (Becton Dickinson, Heidelberg, BRD).

Example III.1

Effect of the fusion construct on active experimental autoimmune encephalomyelitis (EAE)

This model covers a relative broad spectrum of the pathogenesis including activation of autoaggressive T cells in vivo and various inflammatory stages of the disease within the central nervous system. However the disease is normally limited only to perivascular infiltrations and not associated with primary large scale demyelination.

Groups of Lewis rats (n = 3) were immunized with C1 peptide which span the encephalitogenic domain of guinea pig MBP for Lewis rats from aminoacid 68-88. All rats gained weight for the following 8 days (Fig. 6), followed then by flattening of their weight curve beginning on day 9. Beginning day 9 the control, non-fusion construct treated group exhibited rapid weight loss, developed tail limpness followed by distinct hind-limb weakness and roughing of the fur. A partial loss of the righting reflex and incontinence was also observed. These clinical signs of disease lasted until day 16 when the rats began to recover and to regain weight. Those rats which received intraperitoneal injections of fusion construct showed a flattening of their weight curve starting on day 9 but the treatment resulted in a dramatic reduction in the severity of clinical symptoms. Overall they behaved clinically normal. Only a slight partial loss of tail tonicity was observed which started at day 13 and lasted for 2-3 days with no further increase.

Example III.2

Effect of the fusion construct on T cell mediated transfer EAE (tEAE)

The basis of this model are T lymphocytes recovered from MBP or MBP-peptide immunized rats. The cells are established and maintained in vitro

as antigen specific lines. They are characterized as $CD4^+$, $CD8^-$, TcR $V\beta 8.2^+$, MHC-class II restricted T cells which are able to predictively and reproducibly induce EAE upon transfer in vivo into normal naive recipients. Similar as in the active model tEAE is not characterized by demyelination but focuses on the recirculation and inflammatory effector phase of EAE including pathogenic interactions between the T cells and the blood brain barrier.

Groups of Lewis rats (n = 3) were passively transferred with C1-specific T cells (C1-C9) intraperitoneally. The control group which was not treated with the fusion construct (Fig. 7) developed typical severe signs of tEAE accompanied with severe weight loss beginning at day 2 after cell transfer. The onset of clinical signs began rapidly after day 2 and resemble those of active EAE. The rats recovered around day 6. Those rats which were treated with the fusion construct at three different time points showed only a slight weight loss starting at day 3. However, the clinical signs were greatly reduced to only partial loss of trail tonicity which was observed on day 3-6.

Example III.3

Effect of the fusion construct on transfer experimental autoimmune panencephalomyelitis (tEAP)

In this model S100β-specific T cells derived from immunized rats mediate inflammatory lesions throughout the entire CNS, spinal cord, optic nerve, retina and uvea. Moreover, like in human multiple sclerosis (MS), the eyes are commonly affected showing retinal periphlebitis. Cellular infiltrates are observed perivascular but also in the surrounding parenchym but, generally, there are fewer $ED1^+$ macrophages present as compared to MBP induced EAE. Only mild clinical signs such as weight loss can be observed,. A clinically more severe status appears after injection of Mab 8-18C5 [Linington et al., J. Immunol. 23, 1364 (1993)]. Histologically large confluent foci of demyelination arranged around the infiltrates are present bridging this model to certain cases of human MS.

Groups of Lewis rats (n = 3) were passively transferred with S100β protein specific T cells (LS1). These cells induce a severe inflammatory response in the nervous system which, but only minimal neurologic dysfunction in naive syngeneic recipients (Fig. 8., #1-3). However, clinical signs became ouvert when rats (Fig. 8, #4-6) received anti-MOG monoclonal antibodies at day 5 after the cell transfer. All rats exhibited a complete loss of tail tonicity

lasting 48 hrs. In contrast, rats which in addition to Mab 8-18C5 also received the fusion construct did not show any clinical symptoms and behaved completely normal (Fig. 8, #7-9).

Claims

1. Use of a chimaeric TNF binding protein which comprises a soluble part of the human p55- or the p75-TNF-receptor and all or parts of the constant domains of the heavy or light chain of human immunoglobulin or of a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment of autoimmune diseases.

2. Use according to claim 1, wherein the autoimmune disease is multiple sclerosis.

3. Use according to claim 1 or 2, wherein the chimaeric TNF binding protein comprises the soluble part of the human p55-TNF-receptor.

4. Use according to any one of claims 1 to 3, wherein the chimaeric TNF binding protein comprises all domains except the first domain of the constant region of the heavy chain of human immunoglobulin such as IgG, IgA, IgM or IgE.

5. Use according to claim 4, wherein the human immunoglobulin is IgG, especially IgG1 or IgG3.

6. A method of treating an autoimmune disease in a mammal comprising administering to said mammal a therapeutically effective amount of a chimaeric TNF binding protein or a salt thereof as defined in any one of claims 1 and 3 to 5 which ameliorates the effects of such autoimmune disease.

7. A method as claimed in claim 6 wherein the autoimmune disease is multiple sclerosis.

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FIG. 1

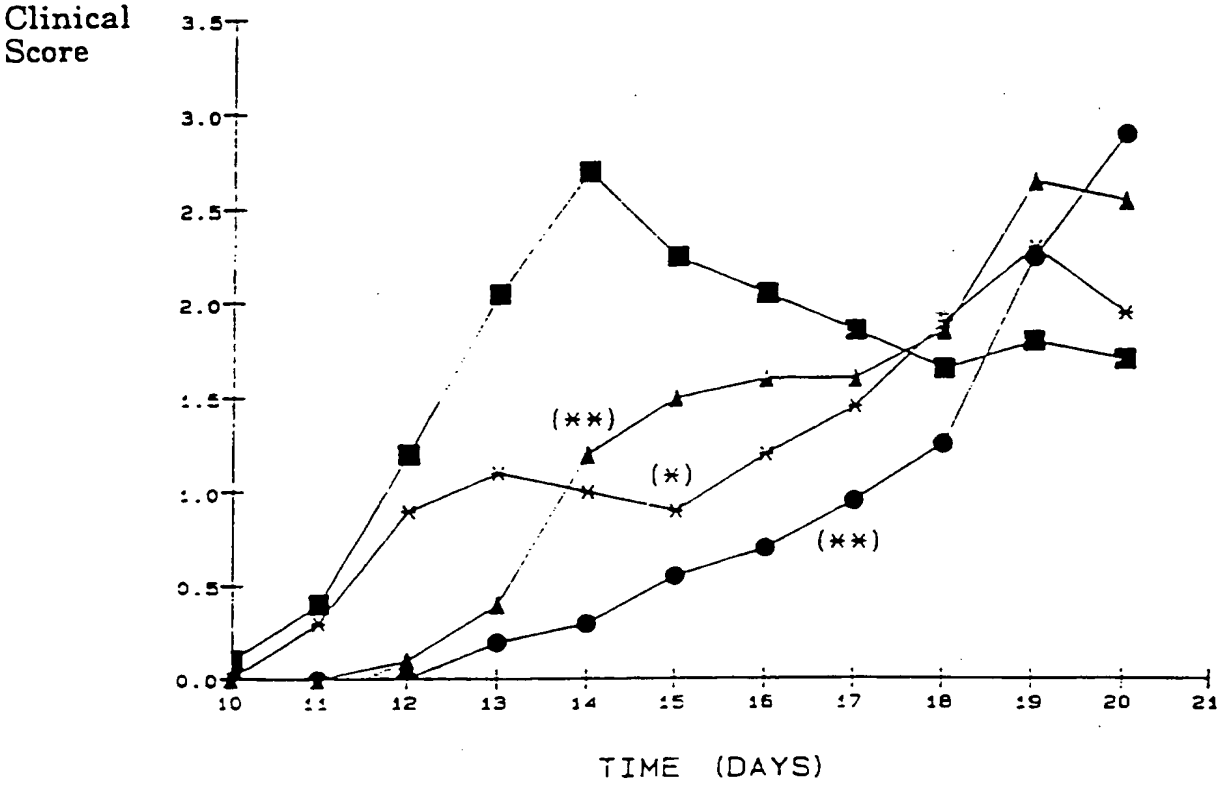


FIG. 2

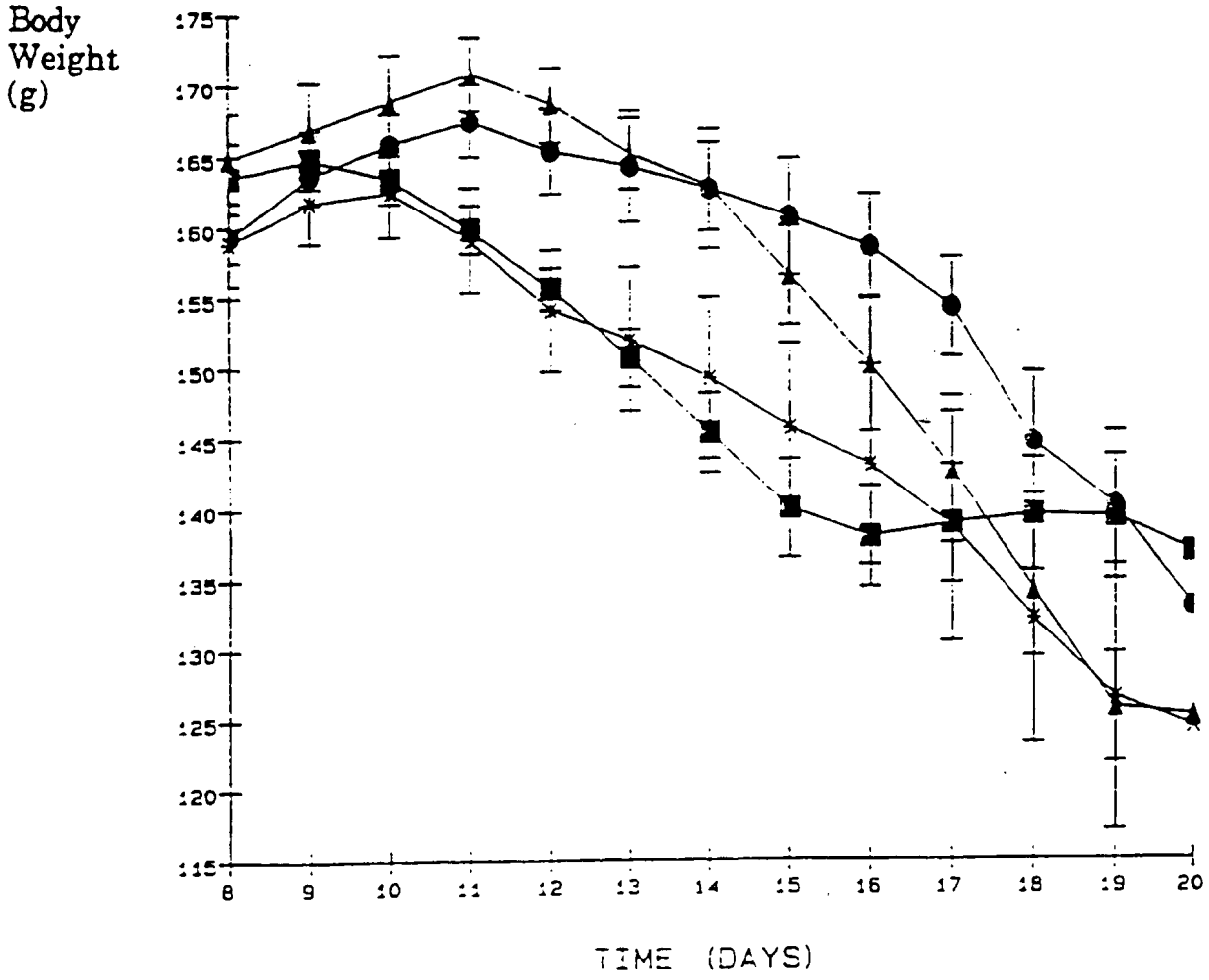


FIG. 3

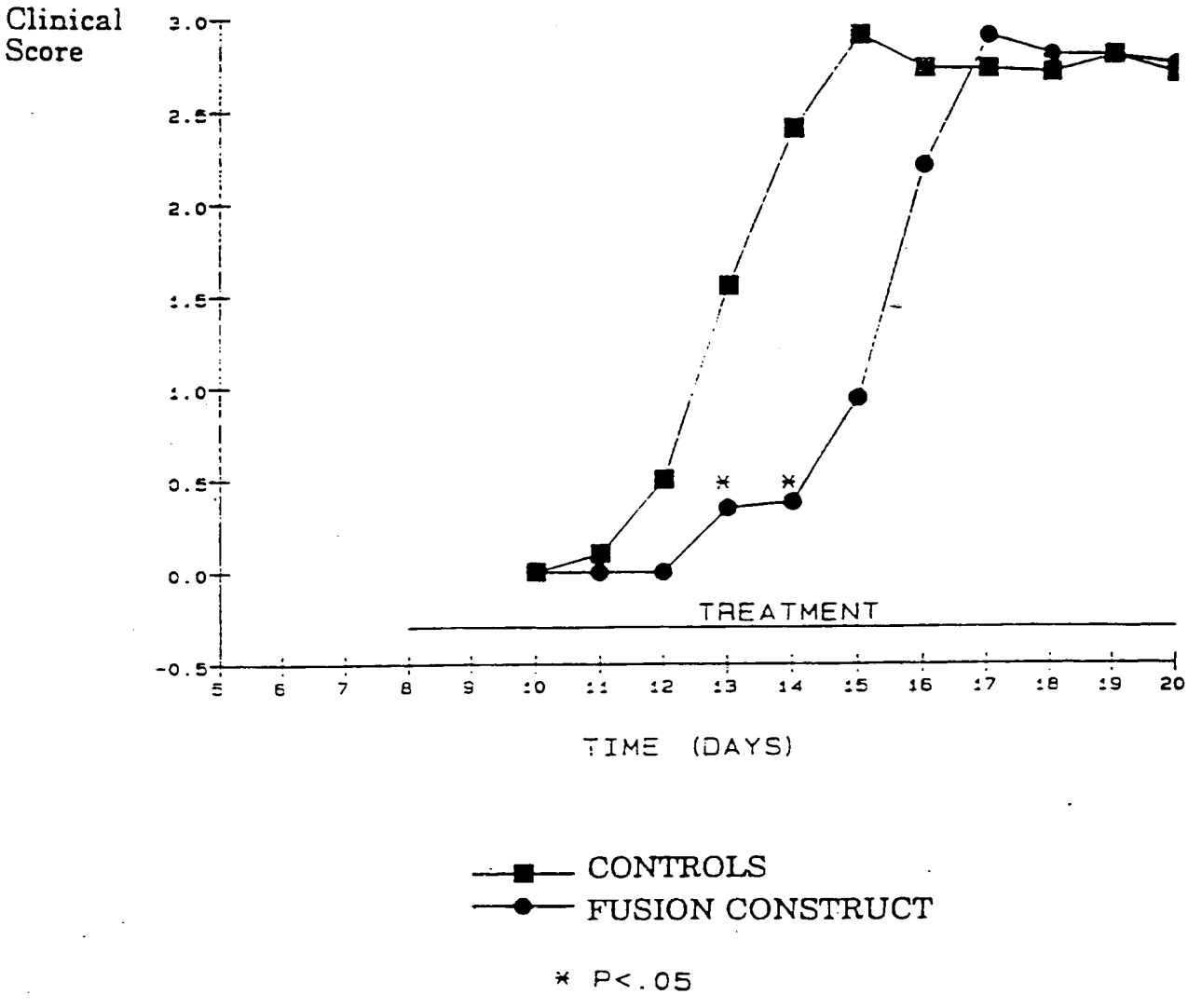


FIG. 4

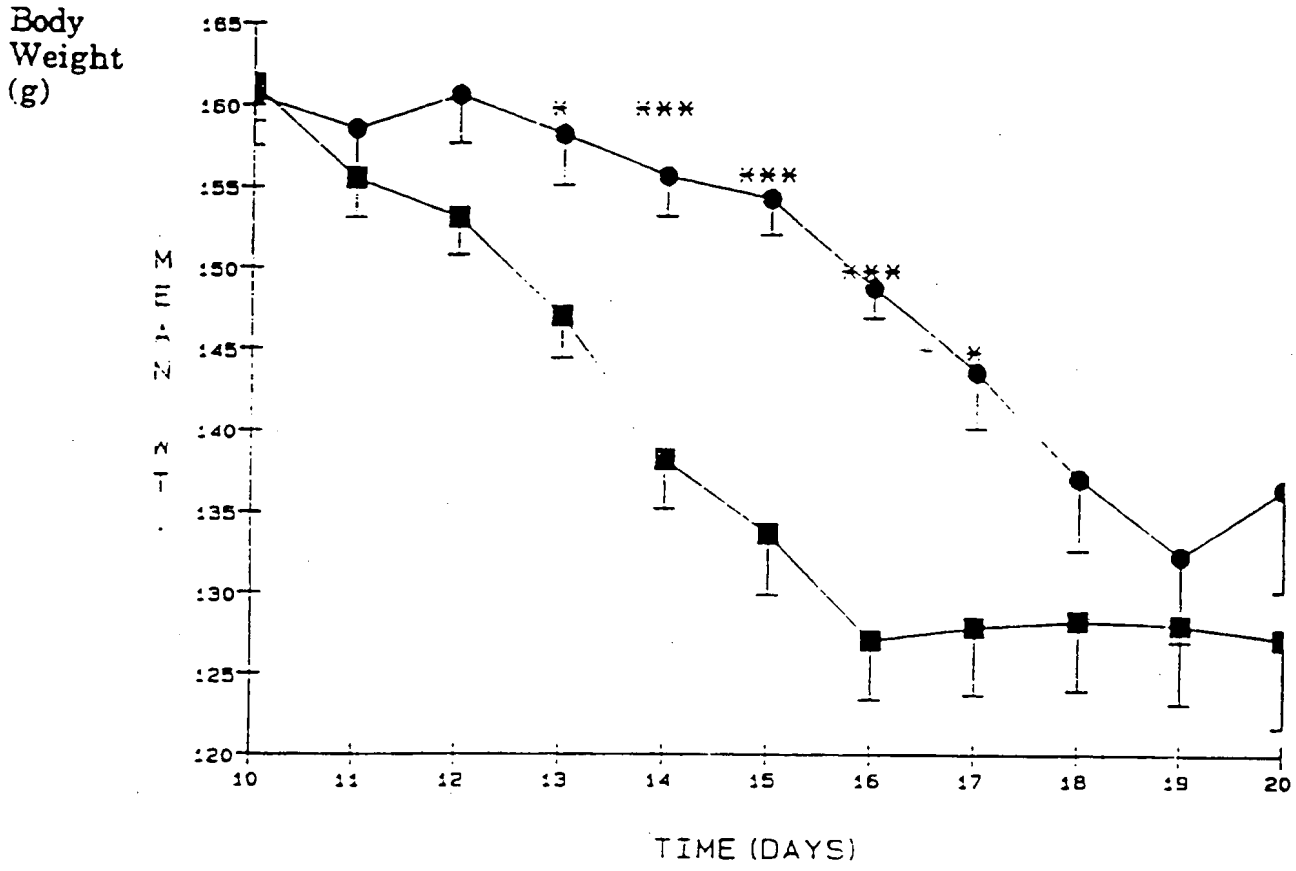


Table 1

Clinical grade		Number of animals during each phase (n=10)					
		Acute		Remission		Relapse	
		Vehicle	Fusion construct	Vehicle	Fusion construct	Vehicle	Fusion construct
Death	(5)	-	1	-	-	2	2
Complete paralysis	(4)	5	1	-	-	6	-
Partial paralysis	(3)	2	-	-	-	-	-
IRR	(2)	2	1	-	-	-	-
Limp tail	(1)	-	1	-	1	-	1
Normal	(0)	1	6	10	8	2	6

FIG. 5a

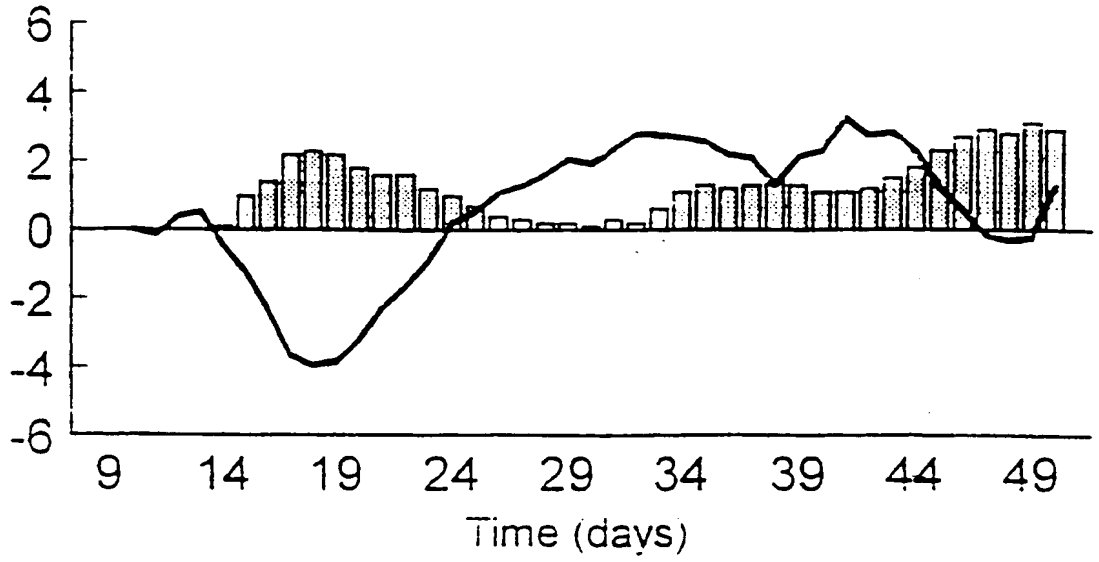
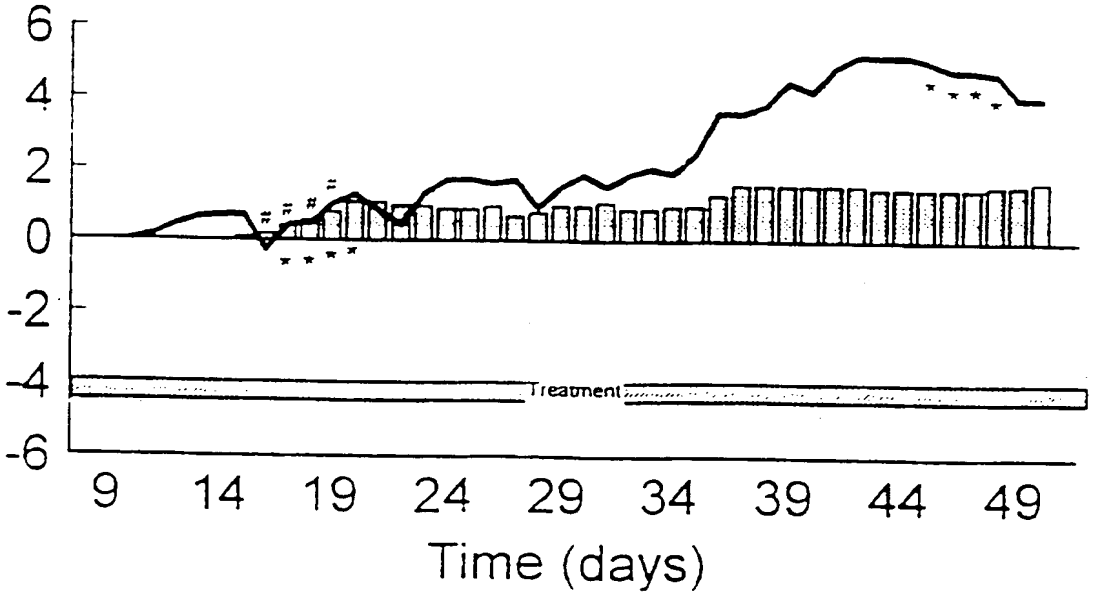
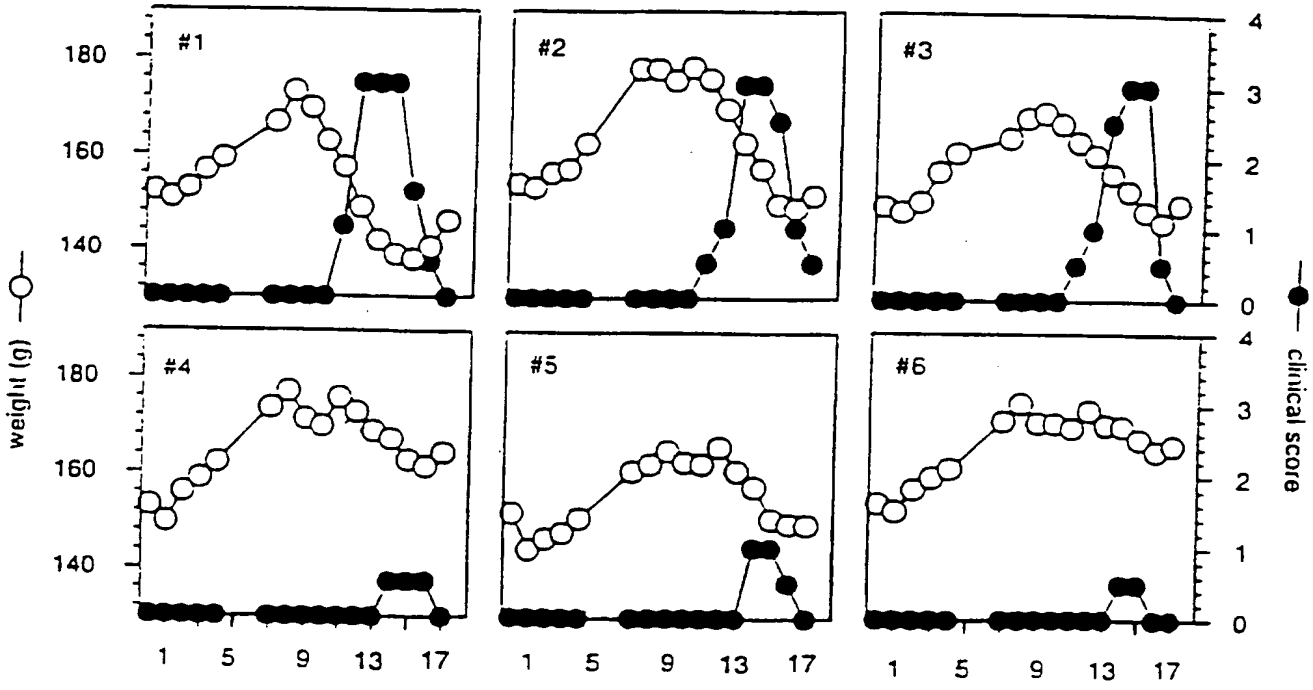


FIG. 5b



Students t-test: * p<0.05 Rank sum: ≠ p< 0.1

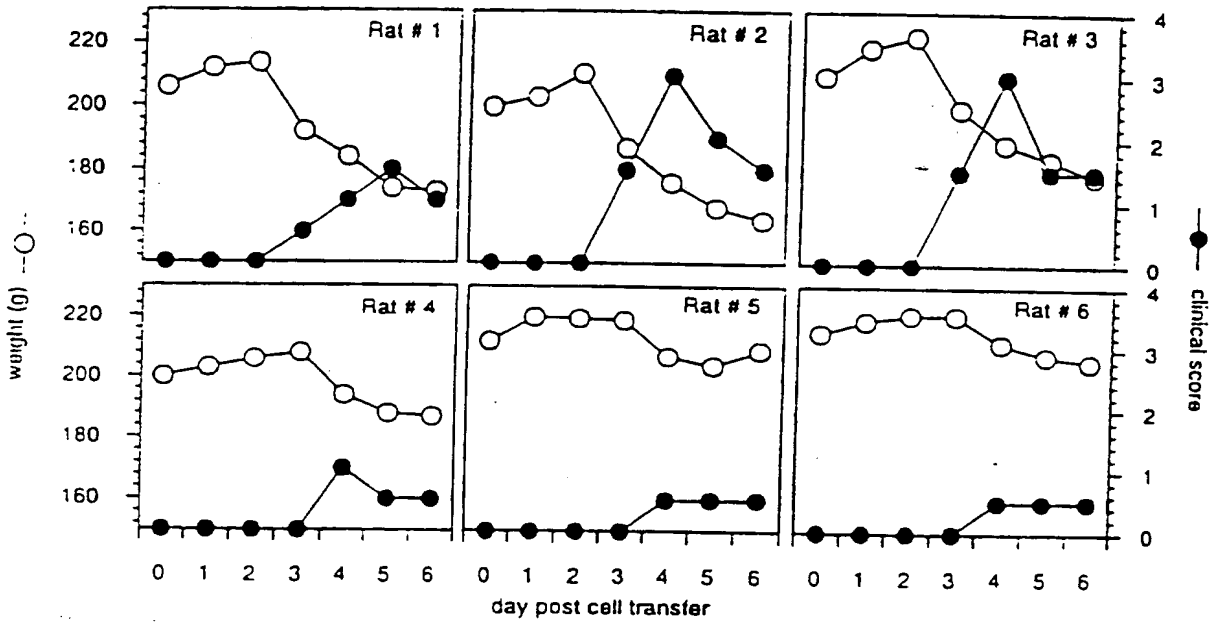
FIG. 6



Immunization : 50 µg C1 s.c. (#1-6)
Fusion construct : 10 mg/kg day 2,7,8,9,10 i.p.(# 4-6)

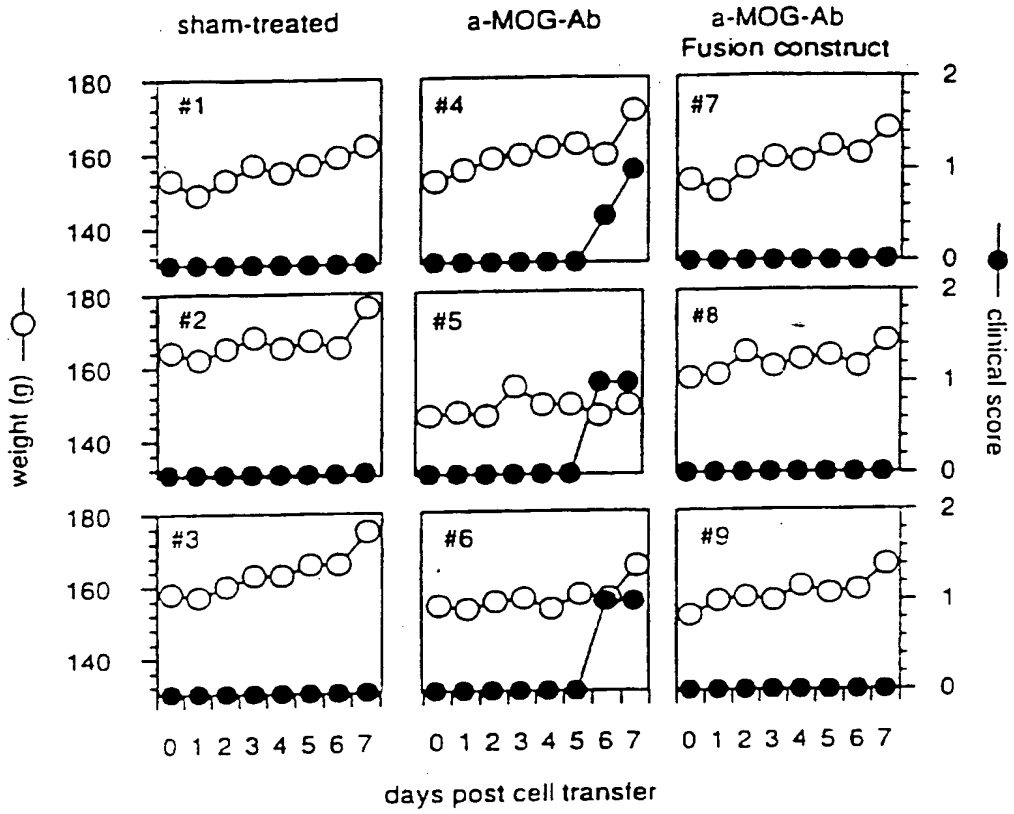
8/9

FIG. 7



Cell Transfer : 6 x 10^6 i.p., Rat 1 - 6
Fusion construct : 10 mg/kg i.p. . day 0, 2, 3; Rat # 4 - 6

FIG. 8



Cell Transfer: 1 x 10^7 LS1 (S100ß-specific), day 0, Rat #1-9

Rat #1-3 : LS1 only

Rat #4-6 : a-MOG-Ab , day 5 (3mg/rat)

Rat #7-9 : a-MOG-Ab, day5; Fusion construct 10 µg/kg, day 1,3,5

INTERNATIONAL SEARCH REPORT

Internat I Application No
PCT/EP 95/02788

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/17 A61K39/395 //C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 08609 (DANA-FABER CANCER INSTITUTE) 28 April 1994 see page 18 - page 19 ---	1,6
X	WO,A,94 06476 (IMMUNEX CORPORATION) 31 March 1994 cited in the application see page 37 ---	1,6
X	WO,A,92 01472 (CELLTECH LIMITED) 6 February 1992 see page 10, line 28 - page 11, line 17 see page 27 - page 28 --- -/--	1,6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 1995	5.12.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat ' Application No
PCT/EP 95/02788

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF IMMUNOLOGY, vol. 151, no. 11, 1 December 1993 BALTIMORE US, pages 6602-6607, WOOLEY P.H. ET AL. 'Influence of a Recombinant Human Soluble Tumor Necrosis Factor Receptor FC Fusion Protein on Type II Collagen-Induced Arthritis in Mice' see the whole document ---	1,6
A	EP,A,0 512 528 (YEDA RESEARCH AND DEVELOPMENT COMPANY) 11 November 1992 cited in the application see the whole document ---	1-7
A	EP,A,0 417 563 (HOFFMANN-LA ROCHE) 20 March 1991 cited in the application see the whole document -----	1-7

1

INTERNATIONAL SEARCH REPORT

Inter. .onal application No.
PCT/EP 95/02788

Box I Observations where certain claims were found unsearchable (Continuation f item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 6-7 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna -l Application No
PCT/EP 95/02788

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9408609	28-04-94	AU-B-	5360294	09-05-94
		CA-A-	2147180	28-04-94
		EP-A-	0671936	20-09-95
WO-A-9406476	31-03-94	AU-B-	4920993	12-04-94
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		EP-A-	0620739	26-10-94
		JP-T-	7504203	11-05-95
		NO-A-	941780	15-07-94
WO-A-9201472	06-02-92	AU-B-	8238191	18-02-92
		EP-A-	0539455	05-05-93
		GB-A,B	2261666	26-05-93
		JP-T-	6501840	03-03-94
EP-A-512528	11-11-92	AU-A-	1609492	12-11-92
		CA-A-	2068027	08-11-92
		JP-A-	5170661	09-07-93
EP-A-417563	20-03-91	JP-A-	4164099	09-06-92